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## CONTRIBUTION TO THE LIFE HISTORY OF CORNUS FLORIDA.\*

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This study of the Flowering Dogwood was undertaken at the suggestion of Professor John H. Schaffner and the first material was collected September 26, 1905. When this material was ready for study, it was found that the flower-buds for the next year had already reached an advanced stage of development. Microspores were already formed and the ovule was far developed. Nevertheless, material was taken at intervals of a week during the fall, monthly during the winter, and weekly again during the spring. Through the kindness of Mr. Robert A. Young material was collected, from June until September, 1906, during the writer's absence from Columbus. To him the author takes this opportunity of expressing his thanks.

Schaffner's weaker chrom-acetic acid solution was employed as a killing fluid. Before placing the head of flower-buds in it, however, the four bracts, which during the winter are tightly folded over them, and in the spring form the conspicuous involucre, were removed in order that the solution could better penetrate the buds. Dehydrating and imbedding were performed in the usual manner. Sections were cut from 8 to 18 microns in thickness. The vast majority, however, were 10 or 12 microns. Analin safranin was first used as a stain and afterwards Delafield's Haematoxylin. The latter proved to be much the better and the best results were obtained by overstaining and then clearing for a long period in acid-alcohol.

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## EARLY STAGES IN THE DEVELOPMENT OF THE FLOWER BUD.

The buds of the same head taken July 21, 1906, showed quite a diversity of stages in development, but the youngest one was practically the very beginning of the flower. The incipient bud arises as a small cylindrical body, from the periphery of the crown of which appear the incepts of the four sepals. These are somewhat transversely flattened cones, the apices of which turn inward (Fig. 1).

In the flower representing the next stage in development, found upon this same receptacle, the incepts of the four petals were seen. These are mere papillae at this time (Fig. 2). They arise at the base of the sepals, on their median side and alternate with them.

A third stage of development was also found upon the same head. In this the four incipient stamens (Fig. 3) take up the same position with respect to the petals that the latter did with reference to the sepals. The petals have become much thickened, while their truncated apices are approaching each other.

The gynoecium has its beginning the latter part of July, as shown in figure 4 of material collected on the 28th. A ridge-like ring develops at the base of the stamens. This grows upwards forming the style with a definite styler canal. In addition to this it may be noted that the apices of the petals have met. These tightly joined petals thus serve as a protection during the winter season.

All of the flowers on a single receptacle collected August 5th showed no further progress in development. Some were even younger than those of the last period.

Flowers of August 11th showed a general development. This was especially manifested in the elongation of the four sets of organs. In addition to this the stamens were becoming differentiated into anther and filament. From the sides of the lower portion of the styler canal, arise the two incipient ovules (Fig. 5).

Sections of flowers collected August 18th show (Fig. 6) that the basal portion of the original, cylindrical bud has become differentiated as the ovulary. The incipient ovule has bent upon itself and is now growing upwards forming the anatropus type.

## DEVELOPMENT OF THE MALE GAMETOPHYTE.

As has already been stated, the stamens begin to be differentiated into a filament and anther as early as August 11th. Internal development has been alike active and the hypodermal archesporial cells of the incipient anther are enlarging. From this time until the next date, August 18th, progress is even more rapid. The archesporial cells have not only divided into the primary parietal and primary sporogenous layers, but these have

in turn divided until there are four parietals, and at least two radial sporogenous layers.

Material collected August 26th showed that in the youngest flowers the three outer parietal layers remain thin and flat while the inner has enlarged and functions as the tapetum. The sporogenous tissue has reached the microsporocyte stage, the nuclei being in synizesis (Fig. 8). Very commonly the chromatin is in a contracted mass on one side of the nuclear cavity while the nucleolus lies free on the other side.

In the oldest flowers of this date the tapetal layer is much broken up into individual cells which are binucleate. The microsporocytes have divided twice in rapid succession without forming a cell wall between them. The result is the spore tetrad within the microsporocyte wall (Fig. 9).

On September 2d the cells of the tetrad had separated into the individual spores. The microspores are somewhat elongated with three double ridges upon their surface.

Commencing September the 8th a gelatinous mass is forming within the microsporangia. On September 24th it had grown much more dense and on the 26th of the same month of the previous year the same condition was present. As this matrix (Fig. 12) remains within the sporangia throughout the winter it no doubt functions as a protection to the microspores and pollen grains. In the spring it begins to dissolve in some sporangia the last of February while in others it remains until the last of April.

At just what date the microspores divide to form the two-celled pollen grain (male gametophyte) was difficult to determine because of the deep stain which the gelatinous matrix takes. It was found, however, that the two-celled stage was present December 4th. In this stage, then, they pass the winter, the male gametophyte being developed the fall previous to the blooming of the flower.

#### THE DEVELOPMENT OF THE FEMALE GAMETOPHYTE.

As has been stated, the incipient ovule makes its appearance about August 11th as a papilla on the wall of the stylar canal. It grows downward and then bends upon itself becoming anatropous August 18th. On this same date the single integument makes its appearance. On August 18th, less than one month after the appearance of the first floral organs, the sepals, the hypodermal archesporial cell is much elongated and contains a large nucleus. This cell becomes the megasporocyte (Fig. 14).

One week later, August 26th, two subsequent cell divisions have occurred. The result is the four megaspores (Fig. 15). Of these the three apical ones are small and non-functional, while the other, functional one is five or six times as large. No cell walls were seen between them.

On September 2d the first division of the functional megaspore has occurred. The result is the two-celled embryo-sac (Fig. 16). The three non-functional megaspores are also apparent as they have not completely disorganized at this time.

A process of dissolution of the nucellus begins about this date which makes the study of the embryo-sac almost impossible. The cells of the sporangium wall begin to break down, leaving fragments of cytoplasm and especially of nuclear material scattered around the sac. This is well shown in the four-celled embryo-sac of September 8th (Fig. 17). It will be noted from this figure that only the apical cells remain intact. The drawing does not show the full amount of disorganizing material, as that of the median portion has been left out in order to show better the four nuclei of the embryo-sac.

From material collected September 24th, 1906, it was not possible to make out the cell stage of the embryo-sac. That taken September 26th, 1905, was thought to be the four-celled sac. The ovules of all of the material from October 12th, 1905, to January 29th, 1906 were so dense with disorganizing cells that the stage of the embryo-sacs could not be determined.

Material collected on February 28th contained embryo-sacs in the eight-celled stage. As this is before cell activity begins in the spring it is quite probable that the sac passes the winter in the eight-celled stage. If this is the case then we have the completion of both the male and female gametophytes before the winter rest begins.

Nearly all of the cells of the nucellus are used up by April 30th. This leaves the eight-celled embryo-sac lying within the integument. The two synergids at the apex of the sac are long, slender, pointed cones, and project far up into the micropylar canal (Fig. 20). They are covered by quite a number of irregular longitudinal ridges. This part of the sac is very dense and always takes a deep stain.

On May 7th the epidermal columnar cells of the stigma begin to elongate. By May 14th these same cells are club-shaped. The conducting cells of the stylar canal are cuboidal and are very glandular in appearance.

The eight-celled stage of the embryo-sac persists till about the 21st of May. It has greatly elongated by this time (Fig. 18). The three intipodal cells are very small and lie in the extreme lower end. A very few, widely scattered, nuclei of the nucellus are to be seen in the sac. The definitive nucleus lies close to the egg, just below it in Fig. 18 and by its side in Figs. 19 and 20.

#### POLLINATION AND FERTILIZATION.

Pollen was collected May 15th, 1906, by simply jarring the flower against the slide. This was the beginning of the shedding of the pollen, for microsporangia of May 14th still contained the

pollen. The grains when shed are very much like the two-celled pollen grain of April 30th (Fig. 13), except they are more elongated, being sub-spindle shaped. In 1907, although the season was somewhat late, the shedding period was about over on May 27th. Fertilization was not observed.

Those flowers which were fertilized, however, could easily be distinguished externally by their increase in size over the others. Of the fertilized flowers there were usually from one to three upon a head. Those receptacles which contained no fertilized flowers were soon shed by the formation of cleavage-planes in the peduncles. Great numbers of the heads thus cut off could be gathered under a single tree.

#### ENDOSPERM AND EMBRYO.

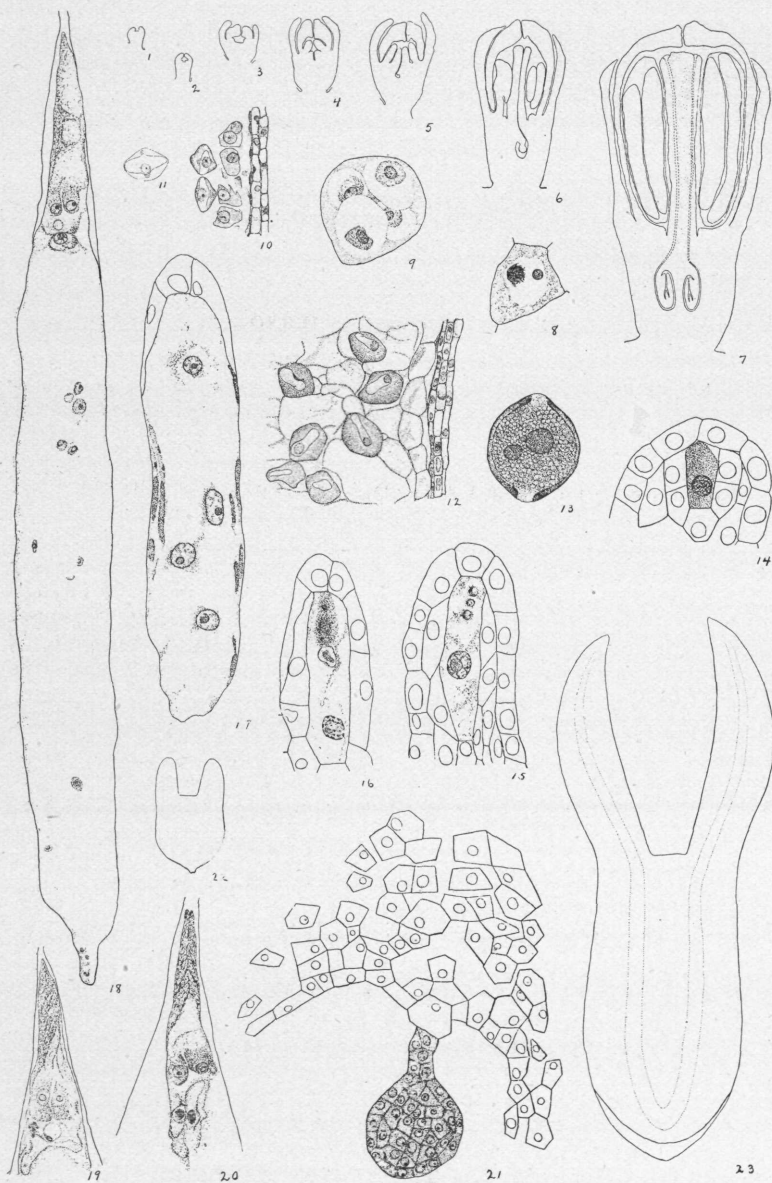
The first material after the last eight-celled embryo-sac (May 21st) was collected June 12th. By this time there had been a rapid development of the endosperm. This filled the upper third of the embryo-sac, extending up to where the synergids lay. The contorted, double pointed cone of the synergids at this time still retains its characteristic shape. The tissue of the integument is beginning to break down.

On June 20th the endosperm had pushed its way in the upper end of the sac practically to the apex of the cone. In the lower half were a large number of loose endosperm cells. By June 29th, the endosperm had reached a very characteristic arrangement. The upper part forms a cap-like structure, the cells of which are arranged concentrically. The integument is nearly destroyed, there being merely strands of disintegrating cell walls.

In the material of June 12th, 20th and 29th, appeared a short chain of irregular cells whose walls were much thicker than those of the cells of the surrounding endosperm. Whether or not these cells were the young embryo was not determined. The first material which, without question, contained a young embryo was taken July 9th. At this time the embryo is a spherical mass of cells suspended by a suspensor from the cap of endosperm cells (Fig. 21). The endosperm cells do not as yet completely fill the embryo-sac and fragments of the integument cell walls remain.

The embryo has developed rapidly by July 21st. On this date the two cotyledons are present (Fig. 22). Some of the cells are becoming differentiated to form the vascular cylinder. The endosperm completely fills the sac except for the small cavity in which the embryo lies.

The last section was made from material collected July 28th. In this the embryo had become quite large (Fig. 23). The cotyledon, hypocotyl, root tip, root cap, and embryonic tissues were well differentiated. The endosperm, as on July 21st, completely filled the sac.



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A complete embryo was taken from a seed of August 11th. The cotyledons had become much expanded, were much broader than the hypocotyl or stem, and about the same in length.

So far as the writer's knowledge goes very little special work has been done on the Cornaceae. Among the related forms, Ducamp<sup>1</sup> has studied certain Araliaceae and Coulter and Chamberlain<sup>2</sup> give a number of observations on *Sium*. By some the Rubiales are regarded as close relatives of the Umbellales. Lloyd<sup>3</sup> has made extensive studies on the embryology of this order; but until we have a more detailed knowledge of the related groups it would be useless to make any generalization from the present study of *Cornus*.

The writer wishes, in closing, to express his thanks to Professor Schaffner who has gone over all the work and given much needed advice and criticism.

#### EXPLANATION OF PLATE XIV.

The drawings were outlined with the aid of a camera lucida, and the following optical combinations were used:

Figs. 1-7, B. & L., 2 oc.,  $\frac{3}{4}$  obj.

Figs. 8 and 9, 13-17, B. & L.,  $\frac{1}{2}$  oc., 1-12 obj.

Figs. 10-12, 18-21, B. & L.,  $\frac{3}{4}$  oc.,  $\frac{1}{8}$  obj.

Figs. 22 and 23, B. & L.,  $\frac{3}{4}$  oc.,  $\frac{3}{8}$  obj.

- Fig. 1. Longitudinal section of young flower-bud, showing two of the four incipient sepals. July 21, 1906.
- Fig. 2. Longitudinal section of a somewhat older flower-bud of the same head, showing the incipient petals.
- Fig. 3. Longitudinal section of a still older flower of the same head, showing, besides the growing sepals and petals, two of the four incipient stamens.
- Fig. 4. Longitudinal section of a flower-bud, to show the growth of the ring-like ridge to form the stylar canal of the carpel. July 28, 1906.
- Fig. 5. Longitudinal section of a flower-bud, to show the growth of the incipient ovule from the side of the stylar canal. August 11, 1906.
- Fig. 6. Longitudinal section of a flower-bud, to show the curving of the lower fourth of the stylar canal around the ovule at right angles to the curvature of the ovule. August 18, 1906.
- Fig. 7. A somewhat diagrammatic longitudinal section of a flower, showing the four sets of organs in position. The two ovules lie in two parallel planes at right angles to the rest of the drawing. The two stylar canals are more or less connected as shown by the dotted portion. January 29, 1906.
- Fig. 8. Section of microsporocyte with massed chromatin and large free nucleolus. August 26, 1906.
- Fig. 9. Section of a microspore tetrad. August 26, 1906.
- Fig. 10. Longitudinal section of part of an anther, showing the epidermis, endothecium, parietal layers, and broken tapetal layer. September 26, 1905.

<sup>1</sup> Ducamp, L. Recherches sur l'embryogenie des Araliacées. Ann. Sci. Nat. Bot. VIII, 15. 311-402. pls. 6-13. 1902.

<sup>2</sup> Coulter, J. M. and Chamberlain, C. J. Morphology of Angiosperms. 1903.

<sup>3</sup> Lloyd, F. E. The Comparative Embryology of the Rubiaceae Bull. Torr. Bot. Club 28 : 1-25. pls. 1-3, 1899. Do. Mem. Torr. Bot. Club 8 : 27-112. pls. 8-15. 1902.

- Fig. 11. Typical microspore of same date.
- Fig. 12. Longitudinal section of an anther, showing the frothy gelatinous substance about the pollen grains. The anther walls consist of the epidermis, endothecium, two intermediate layers, and the tapetum. January 29, 1906.
- Fig. 13. Section of a two-celled pollen grain. April 30, 1906.
- Fig. 14. Longitudinal section of an ovule, showing the archesporial cell which is the megasporocyte. August 18, 1906.
- Fig. 15. Longitudinal section of an ovule, showing the three small, non-functional megasporocytes and the large functional one. August 26, 1906.
- Fig. 16. A longitudinal section of a megasporangium, showing two-celled embryo-sac with the disorganizing nuclei of the three vestigial megasporocytes. September 2, 1906.
- Fig. 17. The ovule cut longitudinally, showing the four-celled embryo-sac with a few of the many disorganizing nucellar cells shown. September 8, 1906.
- Fig. 18. Longitudinal section of an eight-celled embryo-sac, showing the long dense cone, a trace of the egg nucleus lying just below the synergids, the large definitive nucleus below the egg, the three small antipodals and a few of the disorganizing cells of the nucellus. May 21, 1906.
- Fig. 19. A tip of an eight-celled embryo-sac, showing the contorted ridges on the cone. The egg lies in the median line just below the synergids with the definitive nucleus at its side. May 21, 1906.
- Fig. 20. Another figure, to show the double point of the cone of the embryo-sac, the definitive nucleus lies at the side of the egg in this one also. May 21, 1906.
- Fig. 21. A young embryo with suspensor below the cap of endosperm tissue. July 9, 1906.
- Fig. 22. Outline sketch of a young embryo, showing the cotyledons, root tip and fragment of suspensor. July 21, 1906.
- Fig. 23. Outline sketch of an older embryo with cotyledons, stem tip, root tip and root cap. July 28, 1906.
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